POWELL

The Hydrolysis of Proteids

Chemistry
A. B.
1904

THE HYDROLYSIS OF PROTEIDS

...BY...

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THESIS

FOR THE DEGREE OF BACHELOR OF ARTS
IN CHEMISTRY

COLLEGE OF SCIENCE
UNIVERSITY OF ILLINOIS
PRESENTED JUNE, 1904

UNIVERSITY OF ILLINOIS

May 27th 1904

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Jesse R. Powell (Under Dr. W. M. Dehn).

ENTITLED The Hydrolysis of Proteids.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE

OF Bachelor of Arts in Chemistry.

H. S. Grindley.

HEAD OF DEPARTMENT OF

Hydrolysis of Proteids.

Though the proteids form a class of organic compounds which is easily distinguished from the other great groups, like the fats, the oils, the carbohydrates, et cetera, yet no exact definition of a proteid can be given. This is owing to the limitations of our knowledge of both their composition and their structure. In regard to their composition it can be stated with certainty that the elements, carbon, hydrogen, oxygen, nitrogen, and sulphur are always present and in proportions varying, within definate limits, and but slightly amongst the different proteids. Our knowledge of their structure is very meagre, however, and is derived principally from studies of their decomposition products. These studies show that all proteids are closely associated in regard to both structure and chemical properties. It is not my purpose at this point, however, to discuss the proteids in these particulars. In passing it will suffice to state concerning their structure, that the chief decomposition products are amido-acids of the aliphatic series, and concerning their chemical properties, that the proteids possess no very marked acid or basic properties, but form with both acids and bases, salts in which the affinity for both the acid and base is very weak.

For reasons mentioned above a classification of the proteids is yet very uncertain. Levene suggests a scheme of

classification differing from the methods heretofore in use and based upon the fact that proteids decompose differently, there apparently being two distinct classes. To one belongs those proteids composed chiefly of the lower amido-acids, glycocoll, and alanine, the other components entering into the molecule of these proteids in rather small quantities. To the second class belong the proteids with a markedly high content of basic substances. Though late research and study are tending to favor the above method of grouping it has not yet been worked out in detail, and the following classification of Chittenden is quite complete and is one of the best given at the present time.

Simple Proteids.

Albumins	Serum albumin
	Egg ,,
	Lacto ,,
	Myo ,,
Globulins	Serum globulin
	Fibrinogen
	Myosin
	Myoglobulin
	Paramyosinogen
Albuminates	Acid albumin
	Alkali albumin
Proteoses and Peptones	

Coagulated proteids	Fibrin Other coagulated proteids

Combined Proteids

Chromoproteids		Haemoglobin Histohaematins Chlorocruorin Haemerythrin Haemocyanin
Glycoproteids		Mucins Mucoids
Nucleo proteids	Those yielding para-nuclein	Casein Pyien Vitellin
	Those yielding true nuclein	Nucelopiston Cell Nuclein

Albuminoids

Collagen (gelatine)

Elastin

Keratin and Neurokeratin

Properties of Proteids

There are various reactions which the proteids give as a class, and though all reactions will not hold for every proteid and there are other bodies more simple than proteids which give these reactions, yet they serve to distinguish and to isolate these bodies. Salts of iron, copper, mercury, lead, zinc, and many other metals, especially their chlorides, sulphates, and acetates, as well as all the mineral acids, precipitate the proteids.

Since the proteids are complex organic bases, they are also precipitated by the so-called alkaloid reagents. These reactions must take place in an acid solution since in such a solution the proteids show their basic properties. The principal alkaloid reagents are given in the following table:-

Reagent.	Name of reaction.	Remarks.
Platinic chloride		$H_2PtCL_6 \cdot 6H_2O$
Auric chloride		$AuCL_3 \cdot HCl$
Mercuric chloride		
Potassium Platinum Sulpho-cyanate		$2KSCN \cdot Pt(SCN)_4$
Phospho-tungstic acid	Scherbler's	
Phospho-molybdic acid	Sonnenschein's	Used as Sodium Phospho-molybdic acid solution.

Iodine with Potassium iodide	Bouchardat's	Of little use with proteids
Iodine with Hydroiodic acid	Selmis'	,,
Potassium Mercuric iodide	Mayer's or Valier's	
Potassium cadmium iodide	Marme's	
Tannic acid		One of the best reagents.
Picric acid		
Potassium bismuth iodide	Dragendorff's	
Potassium antimony iodide	,,	
Ferrocyanic acid		Used as CH COOH $K_4Fe(CN)_6 + CH_3COOH$

Precipitates with these reagents are usually flocculent and voluminous. In addition to these precipitants there are the so-called color reactions as follows.

Name.	Reagents.	Color	Remarks
Biuret or Piotrowski	Na or KOH $CuSO_4$	red to violet	Given by compounds having two adjacent groups $CONH_2$
Xanthoproteic or Mulder's	HNO_3 with NaOH or KOH	yellow brown, orange	Due to formation of nitro compounds
Millon's reagent	$Hg(NO_3)_2$ HNO_3	red	Given by hydroxy-benxene derivatives.
Lead sulphide	Na or KOH PbO	brown, orblack precipitate	Due to H_2S split off by alkali
Molisch	Alpha-naphthol Conc. H_2SO_4 By adding alcohol ether or KOH	violet yellow	Furfurol reaction

Name.	Reagent.	Color.	Remarks.
Molisch	Thymol conc. H_2SO_4 on diluting	carmen red green	Furfurol reaction.
Adamkiewicz	Acetic acid Sol. & H_2SO_4	green or violet	" "
Liebermann	Fum. HCl	violet, or blue	" "
Froehde's	H_2SO_4 & H_2MoO_4	dark blue	" "
Petri's	Diaxo- benzene sulphonic acid caustic alkali	yellow	
Reichl's	Alcoholic benx- aldehyde, H_2SO_4 and $FeSO_4$	deep blue	
Schultze's	Cane sugar H_2SO_4 Heat to 60"	blue-red	

In a dry condition the protieds are white or yellow bodies usually non-crystalline, though a few have been crystallized. As to solubility, water dissolves them to a very limited extent, while in salt solutions they are still less soluble. Dilute acids and alkalies dissolve them readily, while concentrated acids and alkalies dissolve them with decomposition. Alcohol, ether, chloroform, benzene and like solvents do not effect proteid bodies. On burning, proteids give off characteristic disagreeable odors like that of burning feathers. The ash consists of metals, sulphates, and often phosphates. On heating, all proteids are coagulated, becoming insoluble, each proteid having its

individual coagulation point. Proteids may also be coagulated by acids, alcohol, formaldehyde, and various other reagents. When treated with acids the coagulated proteid that is formed is an acid albuminate, a salt of the basic proteid with the acid. On the other hand treatment with alkalies gives alkali albuminates, or metallic salts of the acid proteid molecule. If salts of the alkaline earths are used very insoluble compounds are obtained. It may also be stated that various salts as sodium, chloride, and magnesium sulphate can be used to "salt out" proteids,i.e.throw them out of solution without changing or coagulating them.

Structure of Proteids

The exact constitution of proteids is unknown, but as an example we give here the elementary analysis by Michel of crystalline Serum albumin, one of the best known pure proteids.

C	53.08%
H	7.10%
N	15.93%
S	1.90%
O	21.99%

Hofmeister, from the sulphur content and the reactions with Iodine calculated the formula as $C_{450}H_{720}N_{116}S_{6}O_{140}$ which would give a molecular weight of 10166. In various proteids the per cents the elements vary somewhat. Carbon may vary from almost 55% to as low as 52%. Nitrogen may go as high as 19% and as low as 15%; but the sulphur content varies the most being from 0.4% to 2%

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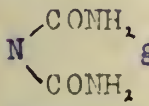
The difficulty of determining molecular weights of these bodies is great, but from freezing point determinations and from the method spoken of above, various results have been obtained varying from 5000 to 17000. These figures show the extraordinary magnitude of the proteid molecule.

As to the combinations in the molecule, little is known, From the decomposition products we know that carbon exists in both the aliphatic and aromatic groups. The greater part (80-87%) exists in combination with nitrogen, for it is so found in the decomposition products; the remainder of the carbon forms nitrogen-free compounds-carbohydrates. However, a portion of the nitrogen, which is easily removed from the molecule, may have been in combination with this latter-mentioned carbon.

None of the decomposition products of the proteids are unsaturated compounds, except of course the aromatic groups. As to the nitrogen, it must exist in the form of primary, secondary, or tertiary amines and not as nitroso, nitro, or azo nitrogen, because of the ease with which it is quantitatively determined by the Kjeldahl method. As stated before a part of the nitrogen is easily split off, being separated by alkalies, and even by dilute acids. The remainder of the nitrogen is separated as monoamido-acids, or as the basic diamido acids. There is nothing to prove the existence of heterocyclic groups in the proteids. The piperazine derivative found by **Cohn** and the pyrrol found

when proteids are fused with caustic alkali, may easily be secondary reaction-products, and the three aromatic amido acids separated are benzene derivatives with amido fatty acids. The biuret reaction tends to show the presence of  $C{<}^{CONH_2}_{CONH_2}$ or -  $N{<}^{CONH_2}_{CONH_2}$ groups. Yet since these reactions are much more pronounced with Albumoses and especially the peptones, perhaps these groups have to be formed by the splitting of some bond.

All proteids, with the exception of a few peptones contain sulphur, which is present in two forms. There is a very loosely-bound hydrogen sulphide derivative which is easily liberated by even cold alkalies, while the remainder cannot be separated by boiling alkalies. This latter mentioned sulphur is detected only on completely burning the substance, when it is obtained as sulphuric acid salts. Just what condition the sulphur is in is not known, but none of the sulphur of mercaptans and compounds in which divalent sulphur is bound to two carbon atoms, as C-S-C is separated when these compounds are heated with lead oxide. On the other hand compounds having the sulphur grouped thus C-S-S-C , and thio-acids do give offat least a part of their sulphur, when so treated. It must be noted however that the group-O-C-S-S-C-O- does not part with its sulphur, the oxygen, directly attached to the carbon, which holds the sulphur, appearing to "fix" the latter. Cystin and cystein also give off

half their sulphur as sulphide when treated with a lead salt and an alkali, but here the action is slow and difficult, contrary to the ease with which sulphur separates from the proteid molecule. However, it has been found that a part of the "loosely bound" sulphur is split from the proteid with much more difficulty than the rest, so pointing to the possibility of cystin-like arrangement of a part of this sulphur. The sulphur decomposition products of proteids so far known are,

Cystin (disulphide of amido-lactic acid)

Thio-lactic acid	Methyl mercaptan
Thio-glycolic acid(?)	Ethyl mercaptan
Ethyl-sulphide	Hydrogen sulphide

Cystin being obtained in far the largest quantities, Drechsel came to the conclusion that the sulphur existed as a diethyl-sulfino-acetic acid or a thetin body. Muller and Seemann came to a like conclusion, but Baumann noticed that cystin easily gave cystein, and this easily broke up into the lactic acid, ethyl sulphide, and hydrogen sulphide as:-

Likewise the mercaptan may easily be derived from the thioglycollic acid, while a thioaspartic acid could be the mother

substance for all the products.

As to various other groups, Low and Lorens have shown the absence of aldehyde and ketone groups in the proteid molecule. There must be present atomic groups which take upon themselves acid or basic properties depending on the presence of acids or alkalies, resulting in the acid or alkali albuminates.

One class of proteids, including Mucins and Mucoids give a glucosamine in large quantities when decomposed. This glucosamine is supposed to come from the breaking down of a higher carbohydrate body which splits off from the proteid molecule earlier in the decomposition. The presence of this body or one very similar has not been proven in the other proteids, with the exception of egg albumen, though in all, a complex body, that may be a carbohydrate, appears to have been found. However no definite ozazones of these bodies have yet been obtained.

Hofmeister and Pick have pointed out the existence of three, more or less distinct groups found in proteids. The so called "Hemigroup", to which the protalbumoses belong, contains little diamido acids, but much monoamido acids, little or no leucine, much tyrosine and skatolamidoacetic acid. They also contain a carbohydrate group and the unknown body tryptophan. Trypsin easily and <u>entirely</u> decomposes this hemi group. The "Anti group" whose representative is the heteroalbuminose, contains much diamido acids, considerable leucin, and other amido fatty

acids, including glycocoll and phenylamidopropionic acid. However, tyrosine, indol derivatives, carbohydrate groups, and tryptophan are lacking. The sulphur, as in the hemi group, is only present as the "loosely bound" sulphur. Both the hemi and anti groups have a higher content of nitrogen and carbon, with a lower content of oxygen than the original proteids. There is little known of the third class, except that it contains a complex carbohydrate-like body, and has comparatively a high oxygen but low nitrogen and carbon content. All three of the groups give the biuret action. Only the third group gives the Molisch reaction. The reactions of Adamkiewicz and Liebermann are given by the hemi and third groups. The hemi group alone gives Millon's reaction, while both the hemi and anti groups give the lead sulphide test, the third group not having been investigated. The xanthoprotein reaction is shown by the hemi group while it is questionable with the other two groups.

Decomposition of Proteids.

In the study of proteids various methods have been devised for their decomposition. However, the most important are as follows:-

Boiling with various mineral acids, more or less concentrated.

Heating with steam at various pressures.

Heating with caustic alkali solutions, with or without increased pressure.

Fusing with alkalies.

Treatment with various animal ferments, especially trypsin.

Action of bacteria.

Action of various ferments of living animals and plants.

In all cases the decomposition is supposed to follow the following course. From the albuminates, and like bodies, the albumoses and peptones are formed, these decompose to form bodies still closely related to proteids, and it is from the final decomposition of these latter bodies that there is obtained the so called crystalline decomposition products of proteids.

The following table gives the chief decomposition products and the methods by which they are obtained.

	HCl	HNO_3	Br	H_2SO_4	Alk.Sol.	Fusion Alk.	Trypsin	Animal ferment	Plant ferment	BACTERIA
Ammonia	#				#		#	#	#	
Carbondioxide	#		#		#			#		
Hydrogen sulphide	#					#		#		#
Glycocoll	#			#						
Amido-valeric acid					#				#	#
Aspartic acid	#		#	#	#		#		#	
Glutaminic acid	#		?	#	#		#		#	
Diamido-acetic acid	#									
Leucine	#		#	#	#	#	#		#	
Lysine	#			#			#			
Arginine	#			#			#		#	

	HCl	HNO_3	Br	H_2SO_4	Alk.Sol.	Fusion Alks.	Trypsin	Animal Ferment	Plant Ferment	Bacteria
Histidine	#			#			#			
Tyrosine	#			#	#	#	#		#	
Cystine	#			#			#			
Alanine					#					
Butalanine					#					
Thiolactic acid	#									
Formic acid					#					
Valeric acid										#
Acetic acid					#					
Caproic acid										#
Butyric acid					#					#
Succinic acid										#
Oxalic acid	#	#	#		#					
Bromoform			#							
Brom-acetic acid			#							
Brom-analine			#							
Ethyl-sulphide	#									
Ethyl mercaptan						#				
Methyl mercaptan						#				#
Indol						#				#
Skatol						#				#

	HCl	HNO_3	Br	H_2SO_4	Alk.Sol.	Fusion Alks.	Trypsin	Animal Ferment	Plant Ferment	Bacteria
Skatol carboxylic acid										#
Skatol acetic acid										#
Phenyl alanine	#								#	
Phenyl propionic acid										#
Phenyl acetic acid										#
Phenyl ethyl amine										#
P.oxy phenyl propionic acid										#
P. oxy phenyl acetic acid										#
Guanidine									#	
Urea								#		#
Uric acid								#		
Kresol										#
Phenol										#
Derivative of Piperazine	#									
Derivative of Pyridine						#				
Acetone	#									
Glucosamine	#									
Oxymandelic acid										#
Melandonic acid	#		#							
Tryptophan			#				#			

When an oxydizing agent is used with the sulphuric acid the products were; formic, acetic, propionic, butyric, valeric, caproic, and benzoic acids, and aliphatic aldehyde, ammonia, benzaldehyde, various nitriles and a heavy oil containing aromatic acids and alcohols.

An oxydizing agent added to the alkali solutions gave acetic, propionic, butyric, and valeric acids, lysine, histidan, pyrol, and guanidin.

Heating with steam only partially decomposed the proteids giving the albumonisis and peptones.

Grouping these products we have:-

I. The monoamido fatty acids, amidoacetic, -valeric, -caproic-lactic,-succinic,-glutaric,-butyric,-propionic, and -thioacetic acids. The NH_2 group being in the alpha position (except a delta-amidovaleric acid.)

II. The aromatic amido acids, phenyl-amido-propionic, para-oxy-phenyl-amido-propionic, and skatol-amido-acetic acids.

III. The diamido fatty acids, diamido-acetic, -valeric, and-caproic acids, as well as arginine and histidine.

IV. Fatty and aromatic acids,(not amido), including all the lower monbasic acids up to caproic, succinic, glutaric, phenyl-propionic, para-oxy-phenyl-propionic, phenyl-acetic, para-oxy-phenyl-acetic, skatol-acetic, and skatol-carboxylic acids.

V. Fatty acids,(not amido), the corresponding amido-acids not having been found, including beta-oxy-butyric, aceto-acetic acids and the oxidation product, acetone.

VI. Various aromatic bodies, phenol, cresol, benzoic acid, skatol, indol, and pyrrol.

VII. A piperazine derivative, (very likely resulting from a secondary reaction).

VIII. The simple compounds, carbon dioxide, oxalic acid ammonia and hydrogen sulphide.

IX. Substances containing sulphur, mercaptans, ethyl sulphide, thio-lactic acid, thio-glycollic acid, and cystine.

X. The sugar-like body, glucosamine.

XI. Tryptophan.

Of these various products only the following thirteen are thought to be primary decomposition products; the others are supposed to be secondary decomposition products.

Amido-acetic acid.
,, -caproic ,,
,, -succinic ,,
,, -glutaric ,,
Phenyl-amido propionic acid
Oxy-phenyl-amido-propionic acid

Skatol-amido-acetic acid
Diamido-acetic acid
,, -caproic acid
Histidine
Guanidine-amido-valeric acid
Glucosamine

Cystine and a few other sulphur compounds.

These decomposition products behave as follows with the various proteid reagents. The oxy-phenyl-amido-propionic acid gives the Millon's and the xanthoproteic reactions. Mollisch's furfurol reaction is given by the carbohydrate group, while the furfurol reactions of Adamkiewicz and Liebermann are given by both the carbohydrate and oxy-phenyl groups. None of the isolated decomposition products give the biuret reaction. The alkaloid reagents only give marked precipatates with the basic compounds and diamido acids.

The great difficulty in interpreting these results lies in the fact that results are hard to duplicate. That is, not only do different proteids give only a part of these products, and those in varying amounts, but the same proteid treated with different acids as hydrochloric and sulphuric, decomposes in entirely different ways.

It would be well to note the attempts to condense some of these decomposition products to form proteid-like bodies, before going farther. Fisher has been about the only one to work in this line and has had remarkable success in forming the "polypeptides", by the condensation of one or more amido-acids. These compounds resemble in a degree true peptones, and Fisher states that he has succeeded in obtaining a similar substance on partial hydrolysis of proteids, and even suggests the possibility of synthesizing a peptone in this manner. At least these re-

sults tend to show how the amido acids unite in the proteid molecule, i.e. after the type of acid amides.

Of the various methods used to decompose proteids into simple, easily-isolated products, the hydrolysis by means of mineral acids has been productive of the best results. By this method Emil Fisher has been able to do his best work in proteid research. After studying Fisher's hydrolysis of casein, and attempting to duplicate his hydrolysis of gelatine, an effort will be made to decompose gluten and isolate the products in a similar manner.

Hydrolysis of Casein

Cohn had studied casein extensively by hydrolysis but more than half of the resulting products was left in the form of a syrup which could not be crystallized. The crystals obtained were in the form of a mixture from which the various components could be separated only with great difficulty. However, Fisher overcame both of these difficulties by forming the ethyl esters of the acids present, fractionating these esters, then saponifying them and crystallizing out the various amido-acids.

Decomposition of Casein with HCl.

Five-hundred grams of casein were covered with 1.5 liters of HCl(1.9 sp. gr.) and in the course of one and a half hours a dark violet solution was formed. It was then boiled under a reflux for six hours. When cool the separated fatty acids were filtered off.

To separate the glutaminic acid, the residue was evaporated to ~~the~~ three-fourths liters and saturated in the cold with HCl gas, then let stand three days in an ice box. The glutaminic acid separated out as the hydrochloride and was filtered off after the addition of a light volume of ice cold alcohol. Yield, about fifty grams o or ten per cent.

Esterfication of the Amido acids.

The filtrate from the glutaminic acid was evaporated under diminished pressure to a thick syrup. This syrup was dissolved in one and five-tenths liters of absolute alcohol and the solution saturated with dry HCl gas, then heated to boiling under a reflux condenser. The resulting solution was then evaporated to a thick syrup under diminished pressure. The operations just described was repeated until three lots of alcohol were used. This removes the water and forms the ethyl esters of all acids present. The final solution was evaporated in four portions under very low pressure, at a temperature not above forty degrees. To separate the esters the thick syrup was treated with an equal volume of water. This mixture was cooled with a freezing mixture and enough NaOH added to about equalize the free HCl used. Then a concentrated solution of K_2CO_3 , and quite a quantity of ether. This separates the weaker aspartic and glutiminic acids, which are very sensitive to free alkali. After a thorough shaking the ether solution of the esters was poured off and fresh ether added. Now there was

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added enough 33% NaOH solution to neutralize all the HCl, and enough solid K_2CO_3 to make a thick mass; so that the esters were thoroughly salted out. After repeated treatment with ether, the brown ether solution obtained was treated for some time with K_2CO_3 then dried for twelve hours over anhydrous Na_2SO_4, the other common drying agents having somewhat of a saponifying effect on the esters. After the evaporation of the ether the residue of the esters obtained was distilled at a pressure of eight to fifteen mm. over the free flame. Up to 40 degrees only a little alcohol came over, above that the fractions obtained were as follows:-

From 500 g. Casein.			From 1Kg. Casein. (at 10mm)	
40 to 55	10 mm.	7 gr.	40 to 45	14 gr.
55 to 80	9 mm.	13 gr.	55 to 65	14 gr.
80 to 100	9 mm.	97 gr.	65 to 80	25 gr.
100 to 130	9 mm.	27 gr.	80 to 85	163 gr.
130 to 160	9 mm.	29 gr.	85 to 110	18 gr.
			110 to 120	40 gr.
			120 to 130	28 gr.
			130 to 160	8 gr.

From the 500 gr. of Casein there was obtained 280 gr. of matter soluble in ether; in distilling up to 170 degrees (pressure going up to 16 mm.) 200 gr. was obtained, but the last fraction contained much of the decomposition products of the esters. To isolate the amido-acids, the esters in the lower fractions were

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[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]

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saponified by boiling with water, while those in the higher boiling fractions were saponified by boiling with a solution of barium hydroxyde.

EXPERIMENTAL WORK.
Hydrolysis of Gelatine.

About 1KG. of best gelatine, the kind used for bacteriological work, was divided into two portions, placed in two five l. flasks and covered with 1.5 l. of concentrated HCl. With frequent shaking a brown solution was formed in about 1 hr. This solution was then boiled for 6 hrs. under a reflux condenser, the liquid turning considerably darker. The lots were now separately evaporated under diminished pressure to a thick sticky mass in a distilling flask, the temperature at no time rising above 60°. Each of the resulting residues was dissolved in 1.5 l. of absolute alcohol. After some time small needle-like crystals, perhaps glycocoll separated. (These E. Fischer does not mention). The alcohol was saturated with dry HCl gas, heated to the boiling point, then cooled and let stand in an ice-chest for 48 hrs. It was found that a mass of glycocoll-hydrochloride crystals had separated out. After the glycocoll had been filtered off, the liquid was evaporated under diminished pressure and extracted with one and five-tenths l. of absolute alcohol. The whole above mentioned process was repeated, giving three crops of crystals.

Glycocoll separated.

1st lot.		2nd lot.
14.5 grs.	1st crystalization	17.5 grs.
110.0 0 ,,	2nd ,,	165.0
10.0 grs	3rd ,,	0.0
134.5 grs.		182.5 grs.

Total 317 grs.

The liquid from the last crystalization was evaporated under reduced pressure to a thick syrupy mass. An effort was made to fractionate this in an ordinary distilling flask connected with a Bruehl apparatus. The first distillation was unsuccessful because no vacuum better than 25mm pressure could be obtained. As a result the liquid could not be heated without foaming over into the receiver. It was deemed inadvisable to continue the study of the decomposition products of gelatine as worked out by Emil Fischer, so attention was turned to a proteid whose hydrolysis had hitherto not been attempted. The gluten of flour seemed to offer a favorable point for attack, because it is a vegatable product and is easily available.

Gluten.

When wheat flour is made into a stiff dough by adding water, and then kneeded under a stream of water until the starch is washed out, there remains a tough elastic sticky mass which is

called gluten. Beccari first published a description of this body in 1745, and gave to it the name gluten. In 1820 Laddei showed that gluten contains two substances, one soluble, the other insoluble in boiling alcohol. Since then much work has been done on this proteid by various men who came to different conclusions as to the number of distinct proteids present, but nearly all agreed that it was a mixture.

Osborne concluded that there were five proteids in the wheat kernel, and designated them as , gliadin, glutenin, a globulin, an albumin , and a proteose. He also speaks of a proteose-like body apparently distinct but he did not obtain a sufficient amount to study. But of these only the first two properly belong to gluten.

Gliadin is separated from wheat flour or gluten by extraction with hot dilute alcohol. It is insoluble in absolute alcohol and is most soluble in 70% alcohol. It may be precipitated from these solutions by sodium chloride. Gliadin dissolves readily in extremly dilute alkalies and acids, but on neutralization is precipitated apparently unchanged. Gliadin gives the usual proteid reactions. The average data of 25 analses of the gliadin by Osborne are as follows:-

C	52.72%	S	1.14%
H	6.86%	O	21.62%
N	17.66		

Glutenin is much the same as gliadin except that it is only slightly soluble in hot alcohol or in water.However it dissolve in dilute alkalies and acids,though not as readily as gliadin.When dissolved in HCl it gives a yellow solution,which turns violet on standing. The accompanying analytical,(the averages of eight analyses by Osborne) results show how closely it is related to gliadin.

C	52.34%
H	6.83%
N	17.49%
S	1.8%
O	22.26%

Preparation of Gluten

A little over 10.5 Kg of flour were worked into a stiff dough with water in batches of about 2.5 Kg of flour to 1.25 -1.5 liters of water.This dough was allowed to stand from 3 to 6 hrs., for then the gluten appeared to thave collected into a uniform elastic mass, which could be more easily worked than that from without standing. One-fourth of such a batch was worked with the hands under running water until all the starch had been washed out. There remained a slightly yellow and very elastic mass of gluten. To the water used in making the dough a little thymol[1] was added to prevent putrefaction. The separated gluten was kept under an ice cold solution of thymol until all the workings

were collected. A sample was taken for analysis.

Analysis (Kjeldahl)

No.	Proteid
1	23.8%
2	24.4%
3	,, %

These results were not concordant, since it was hard to get a true sample from a substance containing so much moisture(75.6%). In all there was little over4.4Kg. of the moist gluten, and since this was 24% proteid (the remainder being chiefly water) there was a little over one kilogram of proteid for hydrolysis. Analysis of the flour showed 12,03% proteid and since 10.5 kg. was used there would be present something over 1.2 K.g proteid. Considering that no particular care was exercised to obtain a quantitative separation of the gluten, the above results check very well.

Hydrolysis of Gluten.

The gluten obtained was divided into two nearly equal portions and each of these was placed in a 4l. flask, and treated with seven-hundred cc concentrated HCl. This stood for several days with a very slightly apparent tendency of the gluten to go into solution, though it became hard and leathery. Then the liquid was saturated with gaseous HCl.

Soon the gluten began to dissolve and the liquid changed from a pale greenish tint to a purple color. On continueing the treatment with HCl, the liquid became very hot and at the same time grew darker in color, indicating the beginning of hydrolysis. The finely divided gluten would not all dissolve but formed a gelatinous-like mass . This was boiled for 6 hrs. under a reflux condenser, to complete the hydrolysis. During this process the black residue separated from the solution and some of it collected on the condenser tubes. The residue was filtered from the solution and after drying in the air weighed about 358 grs. The filtrate was divided into two portions. One was evaporated under diminished pressure at a temperature below 75°. The other which stood while the first was being evaporated deposited about 15 grs. of crystals and was then evaporated in the same way as the first, under reduced pressure. The residue in each case was a thick sticky mass full of crystals, and could not be filtered. On extracting each of the residues obtained, with one and five-tenths 1. of absolute alcohol, the crystals were found to be insoluble and were filtered off. Yield about three hundred and forty five grs. The alcoholic solution was now saturated with dry HCl and allowed to stand in an ice chest for 48 hrs. 26 grs. of crystals separated. The solution was again evaporated under diminished pressure, saturated with dry HCl, and then allowed to stand in an ice chest.

No crystals separated during the first few days, but on standing two weeks a slight deposit of crystals was formed on the bottom and sides of the flask. The liquid was again evaporated as before, preparatory to fractionation under diminished pressure.

Materials Separated.

I. Black residue in condensers	
II. Black residue from the solution	358 grs.
III. Crystals separated before concentration	15 grs.
IV. Crystals separated after evaporation and extration with alcohol	345 grs.
V. Crystals separated out on saturating alcoholic extract with HCl	26 grs.

The above are the weights of the raw products, none being recrystalized or otherwise purified, except drying in a dessicator containing soda-lime to take up excess of hydrochloric acid.

Study of the substances.

The black residue in the condenser contained the elements carbon, hydrogen, oxygen, nitrogen and sulphur.

The black residue separated from the solution during the process of hydrolysis has often been obtained in the hydrolysis of proteid and is supposed to be due to a secondary reaction. It is an organic pigment and has received the name of melanoidic acid.

The crystals separated from the first solution proved to be an organic acid containing nitrogen, and was probably an amido-acid. Its crystaline structure was not like that of glycocoll. It did not recrystalize well. It was very soluble in water, less so in alchol and ether, and was insoluble in acetone, ligroin in and benzene. The crystals contained ammonium chloride and free hydrochloric acid. They were dried in a dessicator with KOH and soda-lime to remove excess of HCl, and then saturated solutions were treated with salts of Cu, Hg, Hg, Ba, Ca, Pt, Ag, and Pb. (a) Pt gave a well crystalized salt but it was found to be $(NH_4)_2PtCl_6$ (B) with lead acetate, a heavy precipitate of $PbCl_2$ was formed, but the acid itself did not precipitate. However, the filtrate from the $PbCl_2$ deposited prismatic crystals on standing (C) By treating the saturated solution with mercurous nitrate the chlorine was precipitated, then the solution was free from Hg by KOH, was filtered and treated with lead acetate. Crystals separated but they were not as well formed as the above mentioned crystals obtained in (b) nor were the crystals the same. (D) Treating with $AgNO_3$, filtering off the AgCl and evaporating gave crystals, indicating the silver salt of the acid, but this did not crystallize well. The method (b) gave the best crystalline product, and one capable of being recrystallized from hot water, though it was difficult to separate it from the lead chloride. A small quantity was separated, recrystalized and

used for Kjeldahl nitrogen determinations. The data obtained varied between 2-4% nitrogen. As no more of the acid was on hand further study of it could not be made.

The crystals obtained on evaporation and extraction with alcohol were found to recrystalize[1] well from a saturated solution in hot water, but they could not be separated fom the black mother-liquor even by repeated crystilization[1]. The solution was therefore boiled with animal char-coal, filtered, and evaporated until crystals began to form. On cooling a large crop of crystals separated. These were recrystalized, and were found to melt with decomposition at 200 . (Glutaminic acid melts at 206°.) The crystals showed a trace of sulphur and chlorine which account for the low melting-point.

Analyses made of these crystals gave the following data:-

	Found			Theory
	I.	II.	III.	(Glutaminic Acid)
N(Kjeldahl)	9.7%	9.2%	9.42	9.52%
C	43.08			40.81
H	6.14			6.26

There can be no doubt that this crystiline[1] product was glutaminic acid. The 345 grs. mentioned above was found to be nearly all glutaminic acid, therefore, it is by far the most abundant decomposition product of gluten.

The crystals obtained on saturating the alcoholic with HCl proved to be ammonium chloride.

Time did not permit the separation and identification of all the hydrolytic products of gluten. There were indications of the presence of hydrogen sulphide, and a mercaptan and also aspartic acid. However, these were not identified with certainty. To conclude it may safely be held that at least the following substances are decomposition products of gluten.

Glutaminic acid.
Melanoidic acid
Ammonia.

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